

AMENDMENT & RESPONSE UNDER 37 C.F.R. § 1.116 - EXPEDITED PROCEDURE

Serial Number: 09/820,193

Filing Date: March 28, 2001

Title: METHODS TO PREPARE AND USE EPIDERMAL STEM CELLS

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Dkt: 875.029US1

CLEAN VERSION OF PENDING CLAIMS

1. (Amended) A method to prepare isolated mammalian epidermal stem cells, comprising:
- (a) providing a sample comprising mammalian epidermal stem cells, which sample is contacted with an agent that binds DNA in viable cells and an agent that binds non-viable cells;
 - (b) separating the sample into a first population of cells that are the smallest 30% of the cells in the sample and a second population of cells that are larger than the cells in the first population, wherein the first population comprises a substantially pure population of epidermal stem cells; and
 - (c) isolating a substantially pure population of epidermal stem cells from the first population of epidermal stem cells.
2. (Amended) A method to prepare isolated mammalian epidermal stem cells, comprising:
- (a) providing a sample comprising mammalian epidermal stem cells, which sample is contacted with a dye with low or no cellular toxicity that binds DNA in viable cells and an agent that binds non-viable cells;
 - (b) separating the sample into a first population of cells that are the smallest 30% of the cells in the sample and a second population of cells that are larger than the cells in the first population, wherein the first population comprises a substantially pure population of epidermal stem cells; and
 - (c) isolating a substantially pure population of epidermal stem cells from the smaller cells.
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3. The method of claim 1 or 2 wherein the mammalian cells are murine cells.
4. The method of claim 1 or 2 wherein the mammalian cells are human cells.
5. The method of claim 1 or 2 wherein the mammalian cells are primate cells.

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- B2
6. (Amended) The method of claim 1 or 2 wherein the sample comprises mammalian epidermal cells which have been dissociated from non-epidermal cells.
7. (Amended) The method of claim 1 or 2 wherein the agent which binds DNA is a Hoechst dye.
8. The method of claim 7 wherein the dye is Hoechst 33342.
- B3
9. (Amended) The method of claim 1 or 2 wherein the agent that binds non-viable cells is propidium iodide.
10. (Amended) The method of claim 7 wherein the agent that binds non-viable cells is propidium iodide.
11. The method of claim 1 or 2 wherein the separation is performed with a flow cytometer.
12. (Amended) The method of claim 1 or 2 wherein the sample is in a medium which lacks azide.
- B4
13. (Amended) The method of claim 1 or 2 wherein the sample is further contacted with a nuclear-retained label prior to separation.
14. (Amended) The method of claim 1 or 2 wherein the cells in the second have proliferative capacity.
15. Epidermal stem cells isolated by the method of claim 1 or 2.
- B5
16. (Amended) A method to prepare mammalian epidermal stem cells, comprising:

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- B5
- (a) contacting a population of mammalian epidermal cells comprising epidermal stem cells with an amount of a first agent under conditions effective for viable cells to retain the first agent;
 - (b) contacting the population of (a) with an amount of a second agent under conditions effective for non-viable cells to retain the second agent; and
 - (c) separating the population of (b) into a first population of cells which first population represents the smallest 30% of the cells which comprise viable epidermal stem cells and a second population of cells which represents larger cells, which second population does not comprise a substantial portion of epidermal stem cells.
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- 17. The method of claim 16 further comprising isolating the epidermal stem cells.
 - 18. The method of claim 16 wherein the mammalian cells are murine cells.
 - 19. The method of claim 16 wherein the mammalian cells are human cells.
 - 20. The method of claim 16 wherein the mammalian cells are primate cells.
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- B6
- 21. (Amended) The method of claim 16 wherein the sample comprises mammalian epidermal cells which have been dissociated from non-epidermal cells.
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- 22. (Amended) The method of claim 16 wherein the first agent is a Hoechst dye.
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- 23. The method of claim 22 wherein the dye is Hoechst 33342.
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- 24. The method of claim 16 wherein the second agent is propidium iodide.
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- B7
- 25. (Amended) The method of claim 16 wherein the sample is further contacted with a nuclear-retained label prior to separation.

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37 26. (Amended) The method of claim 16 wherein the cells in the second population have proliferative capacity.

27. Isolated epidermal stem cells obtained by the method of claim 16.

[NON-ELECTED CLAIMS 28-38 HAVE BEEN WITHDRAWN FROM CONSIDERATION]

39. (Amended) A method to prepare a substantially pure population of mammalian epidermal stem cells, comprising: separating a sample comprising mammalian epidermal stem cells and epidermal cells which are not epidermal stem cells, which sample is contacted with a Hoechst dye and propidium iodine or pyronidine iodine, into a substantially pure population of epidermal stem cells and into at least one population of cells that does not comprise a substantial portion of epidermal stem cells.

40. (Amended) A method to prepare mammalian epidermal stem cells, comprising: separating a sample comprising mammalian epidermal stem cells and epidermal cells which are not epidermal stem cells, which sample is contacted with a Hoechst dye and propidium iodine or pyronidine iodine, into a population which represents the smallest 30% of the cells in the sample and into a population which comprises larger cells, wherein the population with the smallest 30% of the cells comprises a substantially pure population of epidermal stem cells.